REMARKS

Amendments

Claims 1 and 12 are amended to incorporate the recitations of claims 3 and 14, respectively. Claims 3 and 4 are amended to be consistent with the language of amended claims 1 and 12. Claims 7 and 22 are amended to correct typographical errors. These amendments do not raise new issues requiring further search and/or consideration., and moreover, place the application in at least better condition for appeal. Entry of the amendments is respectfully requested.

Rejection under 35 USC 103(a) in view of Cimpoia et al., Janes et al., and Ferrero et al.

Claims 1-24 are rejected as allegedly being obvious in view of Cimpoia et al. (WO 00/47759), the article by Janes et al., the article by Ferrero et al., and the article by Adler et al. This rejection is traversed.

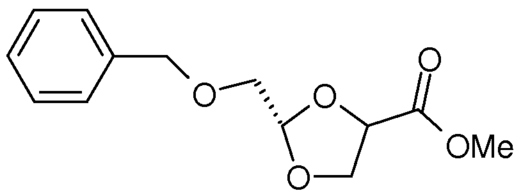
Cimpoia et al. (WO '759) disclose a process for separating ß and α anomers from an anomeric mixture. The anomeric mixture is hydrolyzed with an enzyme selected from cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus lichenformis*, protease from *Aspergillus oryzae*, proteinase from *Bacillus lichenformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus mellus*, proteinase from *Bacillus substilis*, ESL-001-05, pronase protease from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, lipase from *Pseudomonas cepacia,* and bacterial proteinase. See page 7, line 6 - page 8, line 7.

As acknowledged in the rejection, Cimpoia et al. (WO '759) do not disclose use of the enzymes Pig Liver Esterase enzyme or Candida Antarctica "B" lipase In addition, Cimpoia et al. (WO '759) also fail to disclose the use of Candida Antarctica "A" lipase, Candida Lypolitica Lipase, Rhizomucor Miehei Lipase, or Porcine Pancreatic Lipase enzyme.

But, additionally, Cimpoia et al. (WO '759) fails to suggest the use of such enzymes on the substrate recited in applicants' claims. In particular, the disclosure of Cimpoia et al. (WO

‘759) would not suggest to one of ordinary skill in the art the use of, for example, Pig Liver Esterase enzyme or Porcine Pancreatic Lipase enzyme (compare applicants’ claim 1) for resolution of a 2,4 disubstituted dioxolane having two cleavable carbonyl groups.

In Example 1 of Cimpoia et al. (WO ‘759), an anomeric mixture of the compound 2-(S)-benzyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester is subjected to hydrolysis using α-Chymotrypsin. This substrate compound has the following formula:



Thus, in this compound the C-2 group is a benzyloxymethylene group and the C-4 group is a cleavable carbonyl group, i.e., -CO-O-Methyl. Conversely, the compounds of applicants’ formula II have a cleavable carbonyl group at both the C-2 and C-4 positions, i.e., the –O-R2 and –CO-O-R1 groups, respectively. See the definition of R1 and R2 in claim 1.

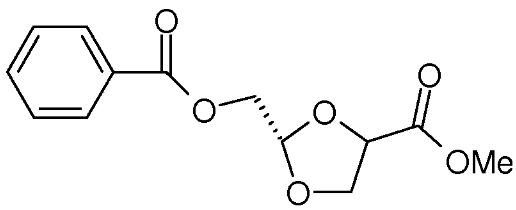
In addition, in Example 1 of Cimpoia et al. (WO ‘759), the process involves separation of the 4(S) and 4(R) isomers while retaining the 2(S) configuration the same. Conversely, the process of applicants’ claim 1 is directed to separation of the 2(S) and 2(R) isomers while retaining the 4(S) configuration the same.

Example 1 of Cimpoia et al. (WO ‘759) teaches the use of Chymotrypsin to resolve 4(S)/4(R) isomers having one cleavable carbonyl group at the C-4 position. But, this Example provides no suggestion of what enzyme would be useful to resolve 2(S)/2(R) isomers having two cleavable carbonyl groups at the C-2 and C-4 position. Such an enzyme would need to act regioselectively (i.e., discriminating between the C-2 and C-4 positions) as well as diastereoslectively (i.e., discriminating between the 2(S) and 2(R) positions) to achieve the desired product.

Examples 28, 30, 32, 34, 36, and 38 are similar to Example 1 in that they involve separating a 2:1(β:α) anomeric mixture of the compound 2-(S)-benzyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester, but use different enzymes then that of Example 1. But, here again, these Examples provide no suggestion as to what enzyme, if any, would be effective, i.e.,

both regioselectively and diastereoslectively, for the separation of the 2(S) and 2(R) isomers (having two carbonyl cleavable groups) while retaining the 4(S) configuration the same.

In Example 4 of Cimpoia et al. (WO '759), a 2:1(β:α) anomeric mixture of the compound 2-(S)-benzoyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester is subjected to hydrolysis using Aspergillus oryzae. This substrate compound has the following formula:



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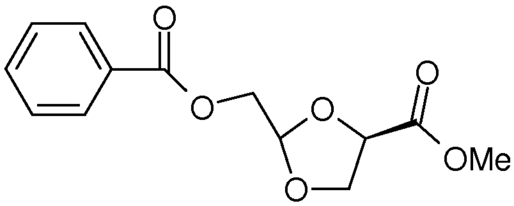
Thus, in this compound the C-2 and C-4 groups are both group cleavable carbonyl groups, i.e., benzoyloxymethylene and -CO-O-Methyl, respectively. However, this Example involves separation of the 4(S) and 4(R) isomers while retaining the 2(S) configuration the same. Conversely, the process of applicants' claim 1 is directed to separation of the 2(S) and 2(R) isomers while retaining the 4(S) configuration the same. Therefore, this example provides no suggestion as to what enzyme, if any, would be effective, i.e., both regioselectively and diastereoslectively, for the separation of the 2(S) and 2(R) isomers while retaining the 4(S) configuration the same.

Examples 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, are similar to Example 4 in that they involve separating a 2:1(β:α) anomeric mixture of the compound 2-(S)-benzoyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester, but use different enzymes then that of Example 4. But, here again, these Examples provide no suggestion as to what enzyme, if any, would be effective, i.e., both regioselectively and diastereoslectively, for the separation of the 2(S) and 2(R) isomers while retaining the 4(S) configuration the same.

In Example 47 of Cimpoia et al. (WO '759), an anomeric mixture of the compound 2-benzoyloxymethyl-4(R)-carboxylic acid-1,3-dioxolane methyl ester is subjected to hydrolysis using α-Chymotrypsin. This substrate compound has the following formula:



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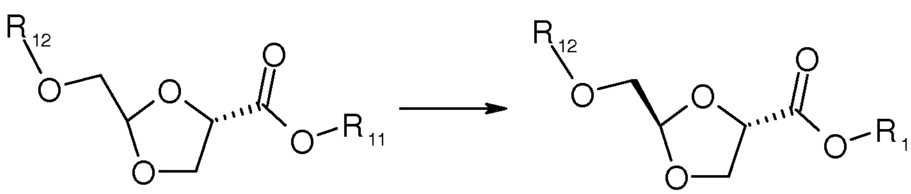
Thus, in this compound the C-2 and C-4 groups are both cleavable carbonyl groups, i.e., benzoyloxymethylene and -CO-O-Methyl, respectively. Thus, this Example involves treating a mixture 2(S) and 2(R) isomers, while retaining the 4(R) configuration the same, to obtain the cis 2(R), 4(R) isomer. Conversely, the process of applicants' claim 1 is directed to enzymatic diastereomeric resolution of the 2(S) and 2(R) isomers while retaining the 4(S) configuration the same. Therefore, Example 47 of Cimpoia et al. (WO '759) provides no suggestion as to what enzyme, if any, would be effective, i.e., both regioselectively and diastereoslectively, for the treatment of the 2(S) and 2(R) isomers while retaining the 4(S) configuration the same. Furthermore, this Example uses α-Chymotrypsin and provides no suggestion of using Pig Liver Esterase enzyme or Porcine Pancreatic Lipase enzyme (compare applicants' claim 1).

In the process of applicants' claim 12 a compound of Formula IV, an anomeric mixture 4(S) dioxolane compounds, is subjected to enzyme resolution (using Candida Antarctica "A" lipase, Candida Antarctica "B" lipase, Candida Lypolitica Lipase, or Rhizomucor Miehei Lipase) to obtain the unhydrolyzed 2(R), 4(S) trans dioxolane compound of Formula III:



IV III .

In Example 1 of Cimpoia et al. (WO '759) Chymotrypsin is used to resolve 4(S)/4(R) isomers having one cleavable carbonyl group at the C-4 position. Thus, the resultant products are 2(S), 4(S) and 2(S), 4(R) products. See Examples 2-3. This Example provides no suggestion of what enzyme would be useful to resolve 2(S)/2(R) isomers having two cleavable carbonyl groups at the C-2 and C-4 position. Such an enzyme would need to act regioselectively (i.e., discriminating between the C-2 and C-4 positions) as well as diastereoslectively (i.e.,

discriminating between the 2(S) and 2(R) positions) to achieve the desired 2(R), 4(S) trans product.

In Example 47 of Cimpoia et al. (WO '759), an anomeric mixture of the compound 2-benzoyloxymethyl-4(R)-carboxylic acid-1,3-dioxolane methyl ester is subjected to hydrolysis using α-Chymotrypsin to obtain the corresponding cis isomer, i.e., 2-(R)-benzoyloxymethyl-4(R)-carboxylic acid-1,3-dioxolane methyl ester. Thus, this Example involves treating a mixture of 2(S) and 2(R) isomers, while retaining the 4(R) configuration the same, to obtain the cis 2(R), 4(R) isomer. This Example provides no suggestion of what enzyme would be useful to treat a mixture of 2(S)/2(R), isomers having two cleavable carbonyl groups at the C-2 and C-4 position, so as to achieve the desired 2(R), 4(S) trans product.

As noted above, the rejection acknowledges that Cimpoia et al. (WO '759) do not disclose use of the enzymes used in applicants' claimed process. However, the rejection asserts that the article by Ferrero et al. discloses that "enzyme-catalyzed reactions have become standard procedures for the synthesis of enantiomerically pure compounds due to their simple feasibility and high efficiency." See page 586 of Ferrero et al. However, this statement merely acknowledges that the use of enzyme-catalyzed reactions in enantioselective synthesis procedures is known. This general statement by Ferrero et al. adds nothing to the disclosure of Cimpoia et al. (WO '759) which already demonstrates the use of enzymes. But, moreover, the statement provides no suggestion for modifying the types of enzymes to be used in the process shown in the examples of Cimpoia et al. (WO '759).

The rejection further states that Ferrero et al. list in Table 1 commonly used enzymes and that this list includes pig liver esterase (PLE), porcine liver esterase (PPL), and Candida Antarctica "B" lipase (CAL). However, the inclusion of these enzymes in this general lists provides no suggestion as to the types or reactions that would use such enzymes, or the types or substrates that could be treated using such enzymes.

In the article by Ferrero et al., reaction procedures using PLE and PPL are illustrated in schemes 12 and 13 (pages 593-594), respectively. In these reactions, the substrates are tri-O-acylated nucleoside analogues having nucleosides bases (or derivatives thereof) attached to the C-4 position of the sugar ring. See also the use of PLE on di-O-acylated nucleoside analogues in

scheme 15 (page 595). Thus, the substrate here does not have a cleavable carbonyl group at the 4-position of the sugar ring. Compare the substrates used in the Examples of Cimpoia et al. (WO '759). These reactions schemes also provide no suggestion of what enzyme would be useful to resolve 2(S)/2(R) isomers having two cleavable carbonyl groups at the C-2 and C-4 position. As noted above, such an enzyme would need to act regioselectively (i.e., discriminating between the C-2 and C-4 positions) as well as diastereoslectively (i.e., discriminating between the 2(S) and 2(R)positions) to achieve the desired product.

In schemes 20-25 (pages 598-604), the substrates are nucleoside analogues having nucleosides bases (or derivatives thereof) attached to the C-4 position of the sugar ring. Thus, here also, the substrate here does not have a cleavable carbonyl group at the 4-position of the sugar ring. Compare the substrates used in Examples of Cimpoia et al. (WO '759).

In schemes 20-25, CAL is used to induce acylation or alkoxycarbonylation of the group at the C2 position of the sugar, not hydrolysis of the group attached to the C4 position of the sugar (i.e., the nucleoside base). Scheme 22 also shows the use of CAL to induce hydrolysis of an acyl group at the C2 position of the sugar. Thus, the disclosure of Ferrero et al. provides no suggestion of what enzyme would be useful to resolve 2(S)/2(R) isomers having two cleavable carbonyl groups at the C-2 and C-4 position. As noted above, such an enzyme would need to act regioselectively (i.e., discriminating between the C-2 and C-4 positions) as well as diastereoslectively (i.e., discriminating between the 2(S) and 2(R)positions) to achieve the desired product. Moreover, the disclosure of Ferrero et al. provides no suggestion of using liver esterase (PLE), porcine liver esterase (PPL), or Candida Antarctica "B" lipase (CAL) in the Examples Cimpoia et al.

The rejection further relies on the article by Janes et al. It is asserted that Janes et al. disclose that dioxolane nucleosides can be antiviral and anticancer drugs. It is further argued that Janes et al. disclose that hydrolytic enzymes are attractive biocatalysts due to "commercial availability, relatively low cost, and tolerance for a wide class of substrates." Additionally, it is argued that Janes et al. disclose a screening procedure at page 201, left column:

> To increase our chance of finding a highly selective commercial hydrolase, we used our previously developed methods to rapidly screen a library of

commercial hydrolases for activity and diastereoselectivity toward the pure diastereoisomers of 2 using a pH indicator in 96 well plates. Our screening has identified two hydrolases, α chymotrypsin and bovine pancreatic proteases, that catalyze hydrolysis of the methyl ester but are sensitive to the configuration of the 2 stereocenter, which lies three bonds away.

The compound 2 referred to by Janes et al. is 2-(R/S)-benzyloxymethyl-4(S)-carboxylic acid-1,3-dioxolane methyl ester. Thus, as with the compound of, e.g., Example 1 of Cimpoia et al. (WO '759), this compound has only one cleavable carbonyl group at the C-4 position. Thus, the disclosure by Janes et al. provides no suggestion of what enzyme would be useful to resolve 2(S)/2(R) isomers having two cleavable carbonyl groups at the C-2 and C-4 position. Such an enzyme would need to act regioselectively (i.e., discriminating between the C-2 and C-4 positions) as well as diastereoslectively (i.e., discriminating between the 2(S) and 2(R) positions) to achieve the desired product.

Thus, Janes et al. disclose the use of α-chymotrypsin and bovine pancreatic protease for separating *cis* and *trans* diastereomers of 2(R,S)-benzyloxymethyl-1,3-dioxolane-4(S)-carboxylic acid methyl ester, wherein the hydrolysis occurs at the carboxyl group. Janes et al. disclose that they discovered these two selective hydrolase enzymes "by screening a library of 91 commercial hydrolases." See the abstract. The *cis* and *trans* diastereomers of 2(R,S)-benzoyloxymethyl-1,3-dioxolane-4(S)-carboxylic acid methyl ester are shown below:

cis trans

In the initial screening test, the diastereoselectivity of the enzymes was estimated by determining their rates of hydrolysis with respect to the individual pure diastereomers, i.e., the *cis* 2(S)-benzoyloxymethyl-1,3-dioxolane-4(S)-carboxylic acid methyl ester and the *trans* 2(R)-benzoyloxymethyl-1,3-dioxolane-4(S)-carboxylic acid methyl ester. See Table 1. Based on

these initial results, Janes et al. selected 6 enzymes for further study, i.e., α-chymotrypsin, bovine pancreatic protease, substilisin from *Bacillus lichenformis*, bovine cholesterol esterase, protease from *Streptomyces caespitosis*, and horse liver esterase. Diversa clonenzyme ESL-001-02 showed moderate estimated diastereoselectivity, but was not selected for further study because it is expensive. See page 9021 right column. It is noted that all seven of these enzymes are among the group of enzymes disclosed by Cimpoia et al. (WO '759). Thus, the disclosure of Janes et al. adds nothing to the disclosure of Cimpoia et al. (WO '759). It is noted that the authors of the Janes et al. article are the same as the inventors of Cimpoia et al. (WO '759).

As noted previously, the disclosure of Janes et al. actually teaches away from applicants' claimed invention. Of 91 enzymes screened, Janes et al. selected only 6 enzymes as warranting further study. Included among the tested enzymes that exhibit insufficient "estimated diastereoselectivity" are pig liver esterase, Candida Antarctica "A" lipase, Candida lypolitica lipase, and Mucor miehei lipase.

The estimated diastereoselectivity is determined as the ratio of cis/trans or trans/cis activity. For the α-chymotrypsin, bovine pancreatic protease, substilisin from *Bacillus lichenformis*, bovine cholesterol esterase, protease from *Streptomyces caespitosis*, and horse liver esterase, the ratio was greater than 8. But, for pig liver esterase, Candida Antarctica "A" lipase, Candida lypolitica lipase, and Mucor miehei lipase, the ratio was 2.09 or lower [pig liver esterase 1.41 (trans) and 2.09 (trans); Candida Antarctica "A" lipase 1.60 (cis); Candida lypolitica lipase 1.4 (trans); and Mucor miehei lipase 1.36 (cis)]. Such low estimated diastereoselectivity suggests away from using these enzymes to separate a diastereomeric mixture.

In the recent Office Action, the Examiner argues that the Janes et al. disclosure does not teach away asserting that "just because the tested enzymes showed insufficient diastereoselectivity towards specific diastereoisomers, would not discourage a person of ordinary skill in the art to test their selectivity towards other diastereoisomers." Applicants disagree.

The diastereoisomers involved are dioxolane compounds. At page 9021 of Janes et al. clearly state that "although 21 hydrolases favored the *cis*-dioxolane, their estimated diastereoselectivites were low, $D<1.1\text{-}6.9$." Based on this result, the authors decided to proceed with six trans selective hydrolases enzymes that had estimated diastereoselectivities greater than

8. Therefore, based on these results, the hydrolases having an estimated diastereoselectivity of less than 8 would not be of interest to a person of ordinary skill in the art seeking to selectively hydrolyze other dioxolane compounds.

See also the disclosure by Janes et al. at page 9023, right column, first paragraph. Here, Janes et al. acknowledged that the "quick *D* values for the six hydrolases were significantly lower, sometimes more than 20 times, than the estimated diastereoselectivities, Table 2." Thus, even for the selected 6 trans selective hydrolases enzymes, the second step in screening process (the quick D measurement) showed that the diastereoselectivity was lower than that suggested by the initial screening results. Thus, this would clearly lead one of ordinary skill in the art away from using the hydrolases that were found to be insufficient in the initial screening tests.

Further, as noted above, the substrate used by Janes et al., 2-(R/S)-benzyloxymethyl-4(S)-carboxylic acid-1,3-dioxolane methyl ester, has only one cleavable carbonyl group at the C-4 position. Since Janes et al. disclose that certain enzymes had insufficient activity with respect to this substrate having one cleavable carbonyl group, one of ordinary skill in the art would be discourage from using such enzymes on a substrate that had two cleavable carbonyl groups.

With regards to applicants' claim 12, the article by Janes et al. is directed to producing the 2(S), 4(S) dioxolane product (i.e., hydrolyzing 2(R), 4(S) dioxolane). There is no suggestion of a process of the preparation of an unhydrolyzed 2(R), 4(S) trans dioxolane compound of applicants' Formula III according to claim 12.

Finally, the rejection relies on the disclosure of Adler et al. to assert that it would be obvious to replace horse liver esterase in the process of Cimpoia et al. (WO '759) with pig liver esterase. The Examiner argues that Adler et al. disclose that pig liver esterase has the same catalytic site as horse liver esterase and that they display similar kinetic properties.

It is noted that Example 32 of Cimpoia et al. (WO '759) involves separating a 2:1(β:α) anomeric mixture of the compound 2-(S)-benzyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester using horse liver esterase. But, as noted above, this Example provides no suggestion as to what enzyme, if any, would be effective, i.e., both regioselectively and diastereoslectively, for the separation of the 2(S) and 2(R) isomers (having two carbonyl cleavable groups) while retaining the 4(S) configuration the same.

The disclosure of Adler et al. is also devoid of any such suggestion. Adler et al.'s disclosure is directed to obtaining a homogeneous preparation of pig liver esterase and to characterize the same. At page 3244, Adler et al. disclose that pig liver esterase and horse liver esterase have the same catalytic site and similar kinetic properties. However, it is not indicated on what substrates this assessment is based, although it is noted that Alder et al. do discuss kinetic measurements based on hydrolysis of methyl n-butyrate.

In any event, the disclosure of Janes et al. already demonstrates that with respect to dioxolane compounds horse liver esterase and pig liver esterase can not be substituted for one another with an expectation of similar results. In the article by Janes et al., the initial screening results showed that horse liver esterase had an estimated diastereoselectivity of 8.52 (trans) and, based on these initial results, horse liver esterase was one of the six hydrolase enzymes selected for further study. But, for pig liver esterase, the estimated diastereoselectivity was 1.41 or 2.09. Such estimated diastereoselectivities clearly suggest away from using pig liver esterase in the process of Cimpoia et al. (WO '759), and clearly demonstrated that one can not substituted pig liver esterase for horse liver esterase in the treatment of dioxolanes and expect similar results.

In view of the above remarks, one of ordinary skill in the art, taking the combined disclosures of Cimpoia et al., Janes et al., Ferrero et al., and Adler et al. would not be lead to modify the process of Cimpoia et al. in such a manner as to arrive at a process in accordance with applicants' claimed invention. Thus, it is respectfully submitted that Cimpoia et al., taken alone or in combination Janes et al., Ferrero et al., and/or Adler et al. fails to render obvious applicants' claimed invention. Withdrawal of the rejection is respectfully requested.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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